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TITLE: Detection of Breast Microcalcifications Under Ultrasound Using Power Doppler and Acoustic Resonance Imaging

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13. ABSTRACT (Maximum 200 Words)

Intraductal breast carcinoma (DCIS) represents approximately one third of mammographically detected breast carcinoma. Currently, DCIS and benign breast microcalcifications can only be reliably be evaluated utilizing x-ray mammography. Our goal with our current project was to utilize breast sonography coupled with the technique of acoustic resonance to image and evaluate the breast microcalcifications in patients prior to biopsy. We have been succesful in visualizing the calcifications utilizing sonography coupled with acoustic resonance. Our analysis, however, showed no statistically significant difference in resonance peaks between malignant and benign types of calcifications in the previous report. In the past year 2003-2004, we have reanalyzed the data to see if there is a statistically significant difference between the benign and malignant types of calcifications. Our reanalysis again does not demonstrate a difference between the two types of calcifications.

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Introduction:

Technical advances in the field of mammography are resulting in higher detection rate of early breast cancers. Some of these technical advances include digital mammography, high frequency ultrasound transducers and breast magnetic resonance imaging (MRI). However, mammography is still the only reliable method of evaluating microcalcifications in the breast. Breast MRI, although has a sensitivity rate nearing 100% for invasive carcinoma, the reported sensitivity for DCIS has been reported to be as low as 40% (1-3). It is estimated that DCIS represents 20-30% of breast carcinomas detected on screening mammography (4) and has been steadily been rising in incidence since the 1970's (5). Since the 1970's the detection rate of ductal carcinoma in situ has steadily increased with the wider use of screening mammography. The detection rate for women less than 50 years of age was reported to be 2.3 cases per 100,000 women in the 1970's increasing to 6.2 cases per 100,000 women by the 1990's (6). More dramatically, in the group of women over the age of 50, the rate has increased from 14.3 to 54.6 per 100,000 (6) in the same time period. We have developed a technique utilizing acoustic resonance to visualize microcalcifications under ultrasound. The concept of acoustic resonance imaging (ARI) is based on the size of the microcalcifications and the binding strength with the surrounding tissues in which it is imbedded. When subjected to a wide frequency range, different sized particles will resonate at different frequencies given the same binding environment. By "tuning" into the appropriate frequency range, it would be possible to selectively visualize microcalcifications of varying sizes. Our goal in this project was to image breast microcalcifications utilizing sonography, which is readily available in breast imaging centers, coupled with acoustic resonance.

Progress Report Body:

The objective of the study was to utilize ultrasound to enhance the detection and evaluation of microcalcifications seen on mammography with power Doppler and acoustic resonance

Methods and Results:

A multi-disciplinary team of researchers at the Department of Radiology, University of Pennsylvania, carried out the proposed work.

Dr. Susan Weinstein is a radiologist specializing in mammography/women's imaging in the Dept. of Radiology. Dr. Weinstein was responsible for the imaging aspect. Dr. Sehgal Ph.D. has expertise in ultrasound imaging. Drs. Sehgal and Weinstein will coordinate the overall study, organize the experimental protocols and carry out computer image analyses. Dr. Weinstein and a research assistant performed color and power Doppler imaging using state of the art imaging equipment and the vibrator prototype. Histology was evaluated by, Dr. Carolyn Mies from the Department of Pathology. The research assistant, Sarah Kangas, was responsible for data management and programming needed for analysis.

At the writing of this report, we are currently at the end of our project.

Task 1: See report from 7/1/00-6/30/02.

Task 2: Patient recruitment period completed

Task 3: Patient recruitment period completed

Task 4: Patient recruitment period completed

Task 5: Data analysis completed Additional data analysis was performed in the past year.

Patient Selection:

The patients presented to the Breast Imaging Section of the Hospital of the University of Pennsylvania on the day of their biopsy procedure. The patients recruited for the study had breast microcalcifications seen on mammography that are considered suspicious. On the day of the procedure, the patients were to undergo excisional biopsy in the operating suite by a surgeon or a percutaneous core needle biopsy in the Breast Imaging Section in order to obtain a tissue diagnosis. Prior to either of these conventional biopsy procedures the patients are recruited for the study.

We had anticipated to enroll about 80 women for the study. There was no specific age range target.

Upon arrival in the department, prior to the breast biopsy, either the radiologist or the research assistant, asked the women if they were interested in participating in a research project. The women were informed that a breast ultrasound would be performed using a small disc that emits vibrations in the sound wave range and that the entire procedure would take approximately 15 minutes. The women were informed that that are no known harmful side effects associated with the procedure but that they would not benefit directly from participating in the study. They were also informed that the results of the sonography would be correlated with the pathology results, and that their identity will not be revealed in any way in future publications. The patients were informed that they may decline to participate. All the elements of the consent form were

reviewed with the patient and a copy of the form was also provided to the patient. If the patient agreed to participate, the consent form was signed and the patient proceeded to have the breast sonography performed by the research assistant and/or radiologist. If the patient declined, she proceeded directly with her planned clinically performed breast biopsy.

Ultrasound Imaging:

Ultrasound and Doppler imaging will be performed using a state of the art,ATL3000 (ATL, Bothell, WA) scanner. This scanner is currently used a research scanner in our department in the laboratory of Dr. Seghal. Microcalcifications, as identified on x-ray mammography, will be imaged by acoustic resonance before tissue diagnosis. Breast sonography will include the conventional breast ultrasound coupled with a disc that has been designed to produce low frequency vibrations. A thin (5-mm) and lightweight (12.9 g) disc vibrator will be held on the breast by surgical tape near the site of calcification identified by mammography. Commercially used ultrasound gel will be used as a coupling medium. The disc vibrator emits vibrations in the sound wave spectrum ranging from 50 to 500 Hz. No side effects are known to be associated with such vibrations. The region of interest was imaged using power Doppler, color Doppler and B scan modes.

The patient then proceeded to have a clinically performed breast biopsy. Tissues from biopsy were processed under routine clinical protocol. The final histology results were obtained and the results correlated with the sonographic findings.

Image analysis:

The videotaped images were subsequently analyzed. Each scan takes approximately 2 minutes. At the video frame rate of 30 images per second, this generates

approximately 120 x 30 = 3600 images. An algorithm was developed to reduce the data by sorting the images on the basis of the frequency of the vibration. These images were subsequently used in a computer program developed in our laboratory to analyze the vibrational response of the microcalcifications. Analysis of color was performed for each image. For each image, computation was performed for mean color level (MCL), percent fractional area of color (FA), color weighted fractional area (WFA). To determine the MCL, the color palate on the image was read by the computer and divided equally on a scale of 0-100. With this scaling system, the computer constructed a look-up table for hue, saturation, and brightness values for the colors in the palette bar. Next the computer identified colored pixels in the image and using the look-up table assigned a color value to each pixel within the region of interest. The color level of the pixels in the region of interest was summed and divided by the number of color pixels to calculate the MCL. The percent fractional area of color (FA) was defined as the area covered by colored pixels divided by the area of region of interest, multiplied by 100%. Color weighted fractional area (WFA) was defined as the (MCL x FA)/100, indicating the presence of net motion within the region of interest. Each parameter was plotted with respect to the frequency range.

Histology Evaluation:

Two representative sections from biopsy tissue samples will be examined for calcifications. The sections will then be fixed in 10% formalin, embedded in paraffin, and sectioned at 5 μm thickness in accordance with standard methods and stained with hematoxylin-eosin (H&E). Calcium phosphate is the predominant form of calcium seen in breast tissue and is

easily recognizable on standard H&E section. On the other hand, calcium oxalate, which can also be present in breast tissue, is particularly difficult to detect on the routine H&E stained sections. However, due to their bipyramidal shape, these crystals are birefringent and will be detected using polarized light.

Results:

Patient accrual was completed by 2002. Eighty five patients with a total of 90 different groups of calcifications were evaluated. Our goal, in this project, was to recuit 80 patients total. Due to technical factors, data could not be obtained from10 patients (12 clusters of calcifications). Additionally, in one patient, the clinical percutaneously biopsy was unsuccesful, therefore, the patient went for an excisional biopsy at another hospital. We did not have the patient's pathology results and she also was not included in the final data analysis.

The age of the patients ranged from 38 to 83 years. The breakdown of the age of the patient population is shown in Figure 1 by the decade. The demographics of the patient population is shown in Figure 2. Out of the 78 different groups of calcifications analyzed, there were 22 cases of malignant calcifications and 56 cases of benign calcifications (Figure 3). Figure 4 shows the age of the patients relative to the pathology results.

First gray scale imaging was performed at the site of the microcalcifications. Next, the same area was scanned in Power Doppler mode in conjunction with acoustic resonance. The acoustic resonance device emitted low frequency vibrations in 10 Hz steps between 50-600 Hz. In the first nine patients, 100 Hz steps were utilized, and it was decided that the steps too large and a decision was made to utilize smaller incremental

steps. The scanning was repeated using 3 different vibration amplitudes to determine variation and the frequency of response of the calcifications to the variation in the amplitude. All the images were stored on a videotape and used for quantitative analysis.

The mammogram from each patient was digitized at 300 DPI and stored in the database for future review. The ultrasound examination was performed using state of the art ATL 5000 ultrasound scanner. All imaging was performed with 12.5 MHz broadband transducer. The instrument resets determined from the studies conducted in the first year were used for all patients.

The acoustic resonance scans involving low frequency vibrations were tolerated by all the patients. We have had no adverse reactions. Average total scan time was approximately 15 minutes. Imaging was completed successfully in 75 patients with 78 clusters of calcifications. In 10 cases (12 clusters of calcifications), due to technical difficulties, data could not be successfully obtained.

We completed quantitative analysis of images from all of the patients as discussed in last year's report. This involved digitizing the images from the videotape. The images of each patients were compiled in a single file and used for quantitative measurement of mean mean color level (MCL), percent area of color (FA) and color weighted fractional area (CWFA). The analysis was performed by selecting the entire image as proposed in the application. However, the images show that structures other than calcifications, such as connective tissue are also enhanced by the acoustic resonance imaging.

Our analysis as reported in last year's report is as follows: Of the 75 patients (78 different groups of calcifications) studied, 56 cases (71.8 %) were benign and the

remaining 22 cases (28.2 %) were malignant. The peak position (P) and the width of the peak at half height (W) were measured for each patient. These values are summarized in Figures 5 and 6. In 19/22 (86.3 %) cases with malignant calcifications, enhancement was seen in the power Doppler mode and a peak in CWFA vs. frequency curve was observed. The mean +/- standard deviation for the peak position (P) was 400 +/- 79 Hz. The peak width (W) was 152 +/-58 Hz. In patients with benign calcifications, the image enhancement was observed in 47 (83.9 %) of 56 cases studied. Nine cases did not show any measurable enhancement. The mean +/- standard deviation for the peak position and peak width were 400 +/- 89 Hz and 144 +/- 58 Hz respectively. There appears to be no statistically significant difference in the peak width and peak position between the benign and malignant calcifications.

Repeat analysis of this data was performed in the 2003-2004 year. The repeat analysis involved reevaluating all the digitized images as discussed above. The repeat analysis took into consideration possible contamination of data due to enhancing "normal" soft tissue structures such as from the surrounding soft tissues and ligaments. We attempted to decrease this contamination by redrawing all the regions of interest (ROI's) as tightly as possible around the enhancement thus decreasing as much of the artifact as possible. In cases with too much artifact, we eliminated the data from analysis. On redrawing the ROI's, 50 biopsy proven benign calcifications and 18 malignant calcifications were reanalyzed. It was felt that in 6 benign cases and 4 malignant cases, too much background noise was present.

Additionally, we noted some of the results showed double peaks in resonance, although the second peak was much smaller than the first in most instances. Additional analysis was also performed taking into account the second resonance peaks.

The peak position was analyzed for all benign and malignant groups of calcifications. If there were 2 peaks, in this portion of the analysis, only the major peak only was used for analysis. (Additional analysis is reported for calculations taking into account the second peak.) The peak position (P) and the width at half height (W) for benign calcifications were: P= 404 +/-144 Hz and W= 144 +/-62 Hz. For malignant calcifications, P= 379 +/- 138 Hz and W=162 +/- 89 Hz. There is no statistically significant difference in the peak width at half height and the peak position between the benign and the malignant groups.

We calculated the peak position and the width of the peak at half height for cases with only one peak. The results are as follows:

Benign (N=29): P = 380 +/- 130 Hz W= 143 +/- 57 Hz

Malignant (N=12): P = 437 +/- 84 Hz W= 196 +/- 90 Hz

There is no statistically significant difference in the peak width and the peak position between the benign and the malignant groups.

Additional analysis was performed taking into account the smaller secondary peak. The following are the results when the peaks are calculated including the secondary peak:

Benign: P=369 +/- 137 Hz W=126+/-62 Hz

Malignant: P=351 +/-139 Hz W=135+/-89 Hz

Again there is no statistical significance in the peak position and the peak width at half height between the malignant and the benign groups. For benign cases, 21 groups of calcifications (42 %) displayed a secondary peak. The secondary peak was seen before the primary peak in 14/21 cases. It occurred after the primary peak in 5/21 times, and approximately at the same position as the primary peak 2/15 cases. For malignant cases, 6 cases (33 %) showed a secondary peak. For malignant calcifications, the secondary peak was seen before the primary peak, 1/6 case and after the primary peak in 5/6 cases.

Additional analysis was performed in the cases with double peaks. We calculated the peak width and the width of the peak at half height for the individual peaks.

	1^{st} peak P (Hz)	1^{st} peak W (Hz)	2^{nd} peak P (Hz)	2^{nd} peak W(Hz)
Benign (N=21)	241 +/-71	73 +/-27	482 +/-79	155 +/-63
Malignant (N=6)	172 +/-18	76 +/-32	360 +/-131	85 +/-45

There is no statistically significant difference in the peak width at half height and the peak position between the benign and the malignant groups for both peaks.

In summary, despite extensive reanalysis of all the data, we could not demonstrate a statistically significant difference between the malignant and the benign groups of calcifications.

Key Research Accomplishments:

- We have been able to visualize calcifications with power Doppler and acoustic resonance in patient studies in 66/78 different groups of calcifications. In 10 cases, moderate amount of background noise was present. No significant enhancement could be seen in 12 cases (9 benign and 3 malignant cases).
- All the visualized calcifications demonstrated resonance peak(s).
- There was no significant difference in the resonance peaks between the malignant and benign calcifications. With further analysis of the data, no statistically significant difference in the resonance peaks could be detected between the benign and malignant calcifications.

Reportable Outcomes:

Weinstein SP, Conant EF, Patton J, Seghal CM. Targeting and core biopsy of breast microcalcifications under ultrasound using acoustic resonance. Radiological Society of North America 1999. (submitted in previous years)

Weinstein SP, Seghal C, Conant EF, Patton JA. Microcalcifications in Breast tissue Phantoms Visualized with Acoustic Resonance Coupled with Power Doppler US: Initial Observations. Radiology 2002;224:265-269. (submitted in previous years)

Weinstein S, Seghal C. Detection of microcalcifications utilizing sonography coupled with power Doppler and acoustic resonance. Era of Hope, DoD Breast Cancer Reasearch Program Meeting 1999.

Conclusions:

Our goal at the start of this project was to determine if we could succesfully visualize calcifications using breast sonography coupled with acoustic resonace. We have been successful in accomplishing this goal in 66/78 groups of calcifications. We also have been successful in our recruiting process. In total, we were able to recruit 85 patients. Due to technical difficulties, we were able to complete the scans in 75 patients with 78 groups of calcifications.

Analysis of the data was then performed to see if there was a difference in resonance between malignant and benign calcifications. Our analysis last year (2002-2003) revealed that no significant difference could be observed between the malignant and the benign calcifications. We speculated that the lack of differentiation between the two types of calcifications may be attributed to enhancement from the secondary structures such as ducts and connective tissues of the breast that would be seen with both benign and malignant calcifications. We have performed additional analysis in this past year (2003-2004) hoping to show a significant difference in the resonance peaks between the benign and malignant calcifications. Extensive additional analysis again does not show a significant difference in the resonance peaks between the malignant and benign calcifications.

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Appendices:

None

List of Personnel Supported by the Grant:

Susan Weinstein, MD – Principal investigator

Chandra Seghal, Ph.D. – Co Principal investigator

Carolyn Mies – pathologist

Sarah Kangas – research assistant (Ms. Kangas replaced Jill Patton)

Fig 1

Age of the Patients

Fig 2

Demographics

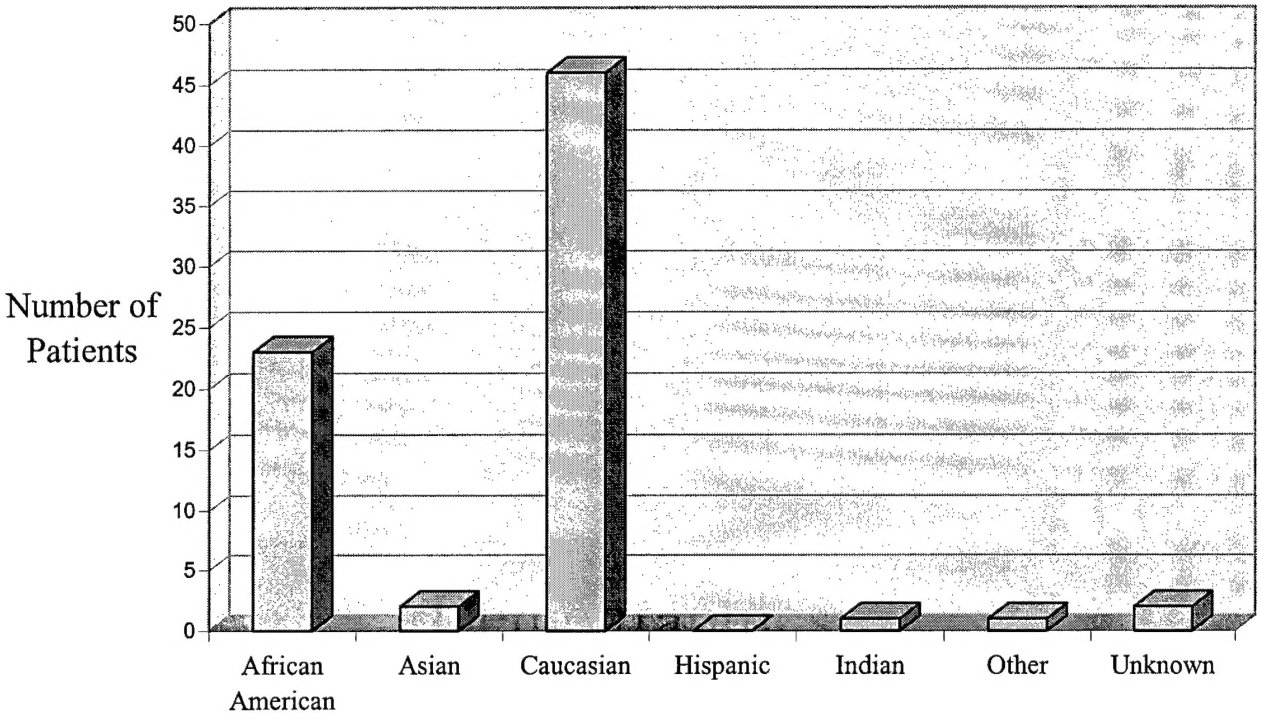

Fig 3

Benign vs. Malignant Calcifications

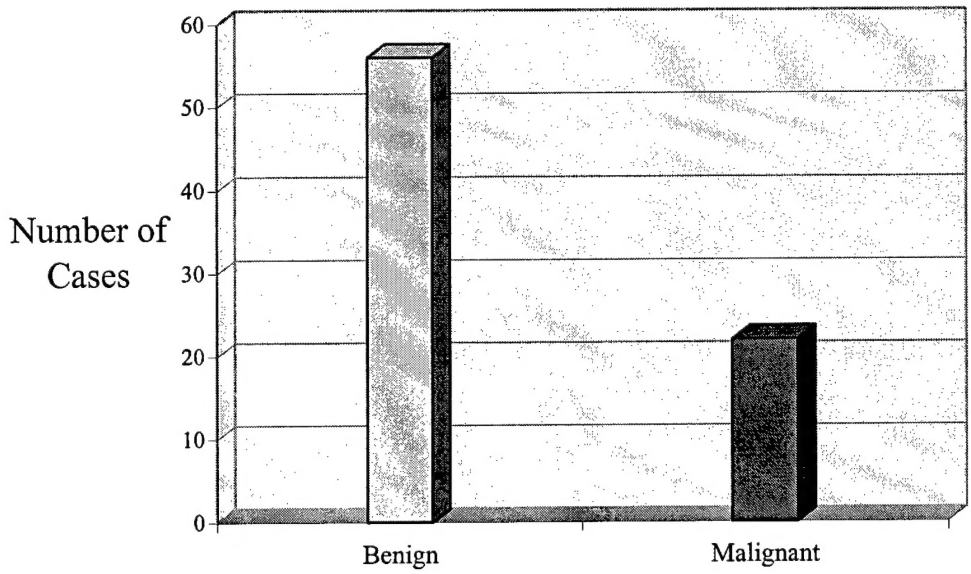

Fig 4

Age of the Patients vs. Pathology

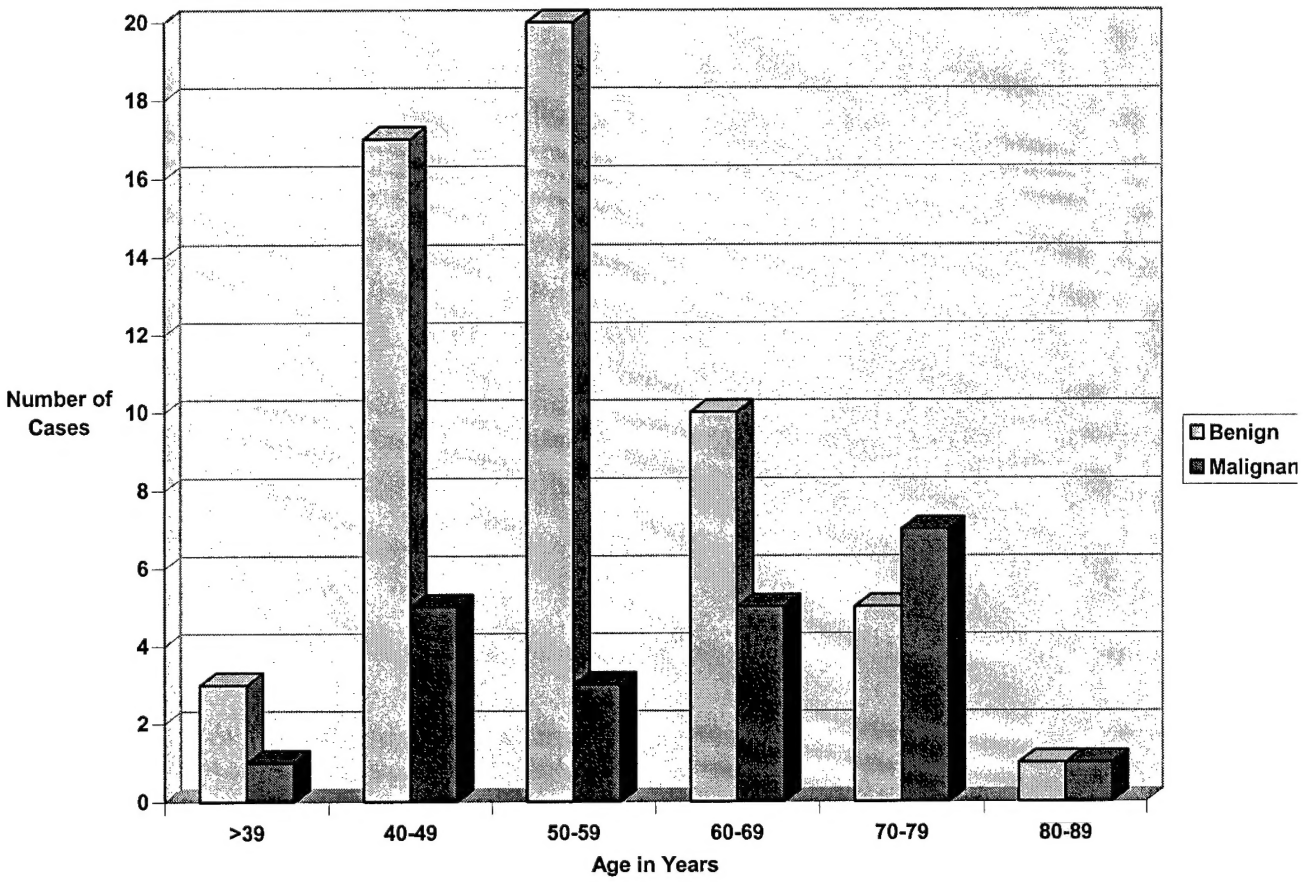

Figure Legends:

Figure 1:
Figure 1 shows the age distribution of the patients who participated in the study. The age of the patients ranged from 38 to 83 years of age.

Figure 2:
Figure 2 shows the demographic distribution of the patients who took part in the research study.

Figure 3:
Figure 3 shows the breakdown of the histology results. There were 78 total groups of calcifications that were successfully analyzed: 22 cases of malignant calcifications and 56 cases of malignant groups of calcifications.

Figure 4:
Figure 4 demonstrates the pathology results relative to the age of the patients. The ratio of benign to malignant calcifications in less than 39-year-old group is 3:1. In 40-49 year old group, it is 17:5. In the 50-59 year old group, it is 20:3. In the 60-69 year old group, it is 2:1. In the 70-79 year old group, it is 5:7. In the 80-89 year old group, it is 1:1.